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Authors: B. [illegible]tmann-Liebold, J. Jauregui-Adell, and H.G. Wittmann.

Title: The Primary Protein Structure of Temperature-sensitive Mutants of Tobacco Mosaic Virus. II. Chemically Induced Mutants. (Die primäre Proteinstruktur Temperatur-sensitiver Mutanten des Tabakmosaikvirus. II. Chemisch induzierte Mutanten.)

Journal: Journal of Nature Research (Zeitschrift fur Naturforschung) Volume 20 b: 1235-12[illegible]9 (1965).

August 1968

SUMMARY

The protein structure of temperature-sensitive mutants of tobacco mosaic virus isolated after treatment with nitrous acid has been determined. The results obtained for 15 mutants, presented in this and the preceding paper, are discussed with relation to the spatial structure of the virus rod.

INTRODUCTION

In the preceding paper (1), the exact location of amino acid substitutions in spontaneously occurring temperature-sensitive mutants of the tobacco mosaic virus (TMV) were described. In the present communication are described protein-chemical investigations on such temperature-sensitive mutants of TMV which were isolated after treatment with chemical mutagens.

MATERIALS AND METHODS

The protein-chemical methods employed in the investigations on mutants described here are identical to those previously given in detail (2, 3).

RESULTS

The mutants described here have arisen either from the TMV strain vulgare or from the spontaneous mutant Al$_4$, and have been isolated after treatment of vulgare or Al$_4$ with nitrous acid. Of the numerous TMV mutants which have been isolated and studied after treatment with chemical mutagens (nitrous acid, hydroxylamine, or 5-fluorouracil), only those which could not multiply at all at temperatures above 30 to 35°C and only slightly at temperatures of 20 to 25°C were employed in these investigations (5). With most other mutants, virus replications is not affected so strongly by temperature. Protein-chemical investigations on these viruses are described in other papers (6).

In the case of the mutants employed here, with the exception of Ni-2516 and Ni-2519, the absent or very weak virus replication at high temperatures is accompanied by the inability of A-protein to aggregate at high temperatures:

if one removes the RNA of the virus and diaggregates the protein core into small subunits, the A-protein will reaggregate into rods in the case of the vulgare strain and many mutants. This protein reaggregation takes place as well at high temperatures as it does at low temperatures in the case of the vulgare strain and many mutants. In the case of the temperature-sensitive mutants, this reaggregation does not occur at all at high temperatures (5). With the exception of the mutants Ni-2516 and Ni-2519, temperature sensitivity and extensive suppression of virus replication at high temperatures can be correlated with at least one amino acid substitution in the virus core protein.

Ni-118: This mutant was isolated after treatment of the TMV strain vulgare with nitrous acid (2). The leaf deformation produced on Nicotiana tabacum var. Samsun is less that produced by the vulgare strain. In contrast to the systemic infection on Nic. tabacum var. Java produced by the vulgare strain, Ni-118 produced a localized infection on this host plant.

After removal of the RNA, if the virus protein is digested with trypsin, the tryptic peptide T1 can be precipitated isoelectrically at pH 4.5 and the supernatant placed on a Dowex-1 Column. The separation and purification of the tryptic peptides is carried out in the manner already described in detail. After three precipitations, the tryptic peptide T1 is separated from associated peptides by Sephadex chromatography, dissolved in urea, and decomposed with iodoacetamide. After removal of the urea and excess iodoacetamide, chymotrypsin digestion (4 hours, 37°C, pH 7.8) is carried out. The chymotryptic partial peptide is passed through a Dowex-1 column and purified by paper chromatography. the experimental details are essentially the same as those employed in previous investigations (2,3).

From analysis of the tryptic peptides (Table 1), it can be seen that the mutant Mi-118 differs from its parent strain vulgare by one amino acid substitution, namely proline ⟶ leucine in the T1 peptide. This substitution can be identified with the No. 80 position by other analytical prodedures carried out on these peptides (Table 1).

A44: Although the spontaneous mutant, A44, is not temperature-sensitive, it should still be described here since from it all the following mutants that are described in this paper have been derived. The knowledge of its protein structure is essential for understanding the investigations on mutants arising from it.

A44 was obtained through a spontaneous mutation from the vulgare strain (7). The amino acid sequence of the tryptic peptides of A44, which are described in previous papers (2,3) was obtained in the same manner as here and differs from that of the vulgare strain by the substitution, isoleucine⟶ threonine, in the tryptic peptide T10. Further investigations on these peptides (Table 2) have localised the amino acid substitution at position 129.

Mi-458: In this case, one is dealing with a mutant which was isolated after nitrous acid treatment of A44 and which produces yellowing on Nicotiana tabacum var. Samsun. The growth retardation resulting from infection and the leaf deformation, however, are generally less than those observed with another yellow mutant, namely flavum.

Analyses of the tryptic peptides showed a single amino acid substitution as compared to A44 - threonine ⟶ isoleucine in the tryptic peptide T3. This peptide was digested with chymotrypsin and the chymotryptic peptide was isolated (Table 3). Through additional protein-chemical analyses on the chymotryptic peptides, T3 and T4, the amino acid sequence substitution can be localised

at position 59 (Table 3 and Fig. 1).

Ni-462: Symptoms do not serve to differentiate this mutant which is derived from A11 from Ni-458 on Nic. tabacum var. Samsun. Mutant Ni-462, which was induced with nitrous acid, can be differentiated from A11 from which it was derived by the presence of two amino acid substitutions: threonine ⟶ isoleucine in the tryptic peptide T1 and serine ⟶ isoleucine in the peptide T3 (Table 4). Both peptides were split with chymotrypsin and the chymotryptic peptides were isolated. As shown in Table 4, in position 5, A11 possesses threonine while Ni-462 has isoleucine, and in position 55, Ni-462 has leucine and A11 has serine.

Ni-1196: Ni-1196 is a mutant isolated from A11 after treatment with nitrous acid and gives a yellowing on Nic. tabacum var. Samsun. It is differentiated from A11 by the amino acid substitution, proline ⟶ serine in the tryptic peptide T4. The substitution has been demonstrated by sequence analysis of this peptide to occur at position 63 (Table 5 and Fig. 1).

Ni-1688: This mutant is derived from A11 (2) and exhibits on Nic. tabacum var. Samsun symptoms which are weaker and clearer as compared to those of the TMV strain. By analyses of all of the tryptic peptides, two amino acid substitutions were found (Table 6): proline ⟶ serine in peptide T4 and proline ⟶ leucine in peptide T12. The substitution in the case of the T4 peptide is at position 63 and in the case of the T12 peptide, it is located at position 156 (Table 6).

Ni-2204 and Ni-2239: In contrast to the mutants described hitherto, the following have been isolated under experimental conditions which permit selection of temperature sensitive mutants (8).

Additional studies on these mutants have shown that they, as expected, are temperature-sensitive, that is, they replicate at an intermediate temperature (23°C) much better than they do at a high temperature (32°C). Also, in vitro,

they can be described as temperature-sensitive mutants: after disaggregation of the virus, the A-proteins are reaggregated only at low and intermediate temperatures but not at the high temperature. These findings suggest that temperature sensitivity in the case of these mutants is related to amino acid substitutions in the core protein in contrast to the temperature-resistant Al4 strain from which they were derived.

This hypothesis was confirmed by the following protein-chemical investigation: in both mutants it was found that the tryptic peptide T1 (Tables 7 and 8) had the substitution serine --- leucine as compared to the corresponding peptide of the parent virus Al4. In the cases of both Ni-2204 and Ni-2239, the substitution occurred at position 15 (Tables 7 and 8). In addition to this substitution, Ni-2204 has still a second substitution, namely threonine --- isoleucine which is located in the tryptic peptide T12. As shown in Table 7, this substitution occurs at position 153.

Ni-2516 and Ni-2519: The isolation (8) of these mutants was accomplished after treatment of Al4 with nitrous acid and was done under conditions which made possible a relatively mild recognition of temperature-sensitive mutants. An Al4 virus preparation treated with nitrous acid was applied to Nic. tabacum var. Xanthi; the plant was incubated at 23°C until appearance of a visable infection, then at a high temperature (32°C), and finally at 23°C. Only those lesions which underwent measurable development at 23°C but not at 32°C were excised, homogenised, and applied to Nic. tabacum var. Samsun. In contrast to all the mutants previously described, with Ni-2516 and Ni-2519, no correlation between the behavior in vitro and that in vivo could be made: whereas both mutants replicated much better at 23°C than at 32°C, the reaggregation of their A-proteins took place only at the high temperature and not at all at low or intermediate temperatures. In this respect, they are different from all the other temperature-sensitive mutants described.

The analyses of the tryptic peptides of Ni-2516 and Ni-2519 showed no differences in the amino acids between these mutants and the temperature-resistant parent strain Al4 (Table 9 and 10; regarding amides, see discussion). The conclusion is thus confirmed that these mutants form a special class of temperature-sensitive mutants. The basis for temperature sensitivity cannot lie in an altered core protein or in a reduced or absent capability of the protein subunits to undergo aggregation at a high temperature. It is suggested, therefore, that in these cases, the temperature sensitivity is caused in the following way: an enzyme necessary for virus replication and directed by the virus RNA is altered by means of an amino acid substitution.

DISCUSSION

In the course of our investigations (2) on the genetic code, the core proteins of some 200 chemically-induced as well as spontaneously occurring TMV mutants have been analyzed protein-chemically. A portion of these mutants are temperature sensitive, and the protein structure of 15 such mutants have been described in this and a preceding investigation (1). The results are shown in Tables 11 and 12. Since as of yet, not all of the 200 TMV mutants analyzed have been tested from temperature sensitivity, additional temperature-sensitive mutants may be found in our material.

The temperature-sensitive mutants so far described can be divided into two groups: in the first group, which by far predominates numerically, the virus core protein, as a consequence of a chemically induced or spontaneously occurring mutation of the virus RNA, differs from the protein of the parent strain in question by the substitution of one to three amino acids. The altered core protein can be used for an organized reaggregation to rods at a low temperature (10°C) but not at a higher temperature (30°), as is the case with the parent strain. This temperature-dependent inability for aggregation

in the case of the mutants of the first group is very likely the basis for the fact that replication at a high temperature (32°C), if present, is significantly lower than that at an intermediate temperature (23°C). The fact that the core protein of these temperature-sensitive mutants is not denatured during the absence of replication can be demonstrated by the fact that virus particles replicating at 23°C can endure temperature treatment at 60°C. The same holds true for the A-protein and 30°C treatment. Similar results to these have been obtained in another system, namely the T4 bacteriophage (9).

In the case of the second group of temperature-sensitive mutants to which Ni-2516 and Ni-2519 belong, the amino acid sequences of all the tryptic peptides are the same as those of the temperature-resistant parent strain. (When indirect amide determinations were carried out, no differences in the the chromatographic and electrophoretic behaviors of the peptides were ascertainable; to be sure, this is not exact enough to exclude amide differences in large peptides such as T1. If amide differences should exist, then they have no influence on the aggregating capability of the protein subunits as in vitro experiments have shown.) As expected, the disaggregated core proteins of both mutants Ni-2516 and Ni-2519, as well as the protein of the parent strain All, reaggregate at low and high temperatures equally well.

The extensive inhibition of virus replication at high temperatures cannot, therefore, be due to the interuption of the aggregation process of protein subunits by high temperature in the case of these mutants. Thus, the following hypothesis is offered: the cistron for the determination of the core protein amounts to about 7.5 % of the TMV RNA if one assumes a triplet code. In the case of treatment with chemical mutagens, not all of the nucleotides that are altered are involved with the virus core protein. Nucleotides may be altered which, among other things, probably determine the protein structure of enzymes which are necessary for virus synthesis. Amino acid substitutions in these

enzymes, as well as in the virus core protein, could lead to functional damage under certain conditions (such as temperature increases) and thereby lead to a partial or complete inhibition of virus replication .

The experiments with temperature-sensitive mutants showed clearly that the substitution of a single amino acid is enough to alter the virus protein sufficiently so that under the appropriate conditions, little or no virus can be synthesized. The following mutants provide such examples: In these cases, the difference in a single amino acid between these and the temperature-resistant parent strain is sufficient to provide complete or partial inhibition of the aggregating capability of protein subunits at high temperatures (table 11): Ni-2239, with the substitution serine → leucine in position 15; flavum with aspartic acid → alanine in position 19; Ni-118 with proline → leucine in position 20; Ni-458 with threonine → isoleucine in position 59; Ni-1196 with proline → serine in position 63; and CP-415 with asparagine → lysine in position 140. In this connection, it is worthy to note that in the case of one of the TMV mutants (10) with two amino acid substitutions (11,12), the protein subunits of the core protein are able to aggregate into rods in an orderly fashion at all temperatures both *in vivo* and *in vitro*.

The ability of a mutant to be temperature-sensitive as well as resistant does not necessarily depend on a single give amino-acid substitution. For example, the mutants Ni-118 and Ni-1927 can be differentiated from each other by the fact that in the former, the substitution proline → leucine occurs in position 20 while in the latter, the defect is located at position 156. One of these mutants (Ni-118) is temperature-sensitive while the other (Ni-1927) is temperature-resistant. The same holds true for other substitutions, for example, threonine → isoleucine in different positions of temperature-sensitive as well as resistant mutants.

In this respect, it is of interest to compare the primary structures of vulgare, flavum, reflavescens, and revirescens (Table 12). If one disregards position 138, which has no significance with regards to the question of temperature-sensitivity (vulgare and revirescens differ from each other only at this position and both are temperature-resistant), then the four mutants differ from each other only at position 19: flavum has alanine; reflavescens, valine; and vulgare and revirescens have aspartic acid. While the first two mutants are temperature-sensitive, the last two are temperature-resistant. In the case of otherwise identical amino acid sequences, the nature of the amino acid at this position is decisive: the presence of aspartic acid at position 19 endows the protein with the property of aggregating correctly at a high temperature. If this amino acid is replaced by alanine or valine, then the aggregating capability is lost and, to be sure, in the case of alanine more strongly than with valine.

Important for the question of temperature sensitivity, that is, the loss of the ability to aggregate correctly under given conditions, is the combination of a given amino acid with a given position in the protein chain. Next to the question of the nature of the substituted amino acid (hydrophilic, hydrophobic, acidic, basic, or aromatic side chain), the position of the substitution within the secondary as well as the tertiary structure of the protein subunit is important; that is, does a particular amino acid in the alpha-helix lie in the vicinity of the subunit surface or more towards the interior; does the new amino acid induce an important linkage for the three-dimensional structure of the protein subunit, etc.

As soon as the three dimensional structure of the protein subunits has been elucidate, the numerous TMV mutants (2,4,6,13,14) for which the amino acid substitutions have been identified, particularly the temperature-sensitive

mutants, will provide , roductive grounds for the correlation of investigations on structural problems with the aggregation and stability of TMV protein sub-units.

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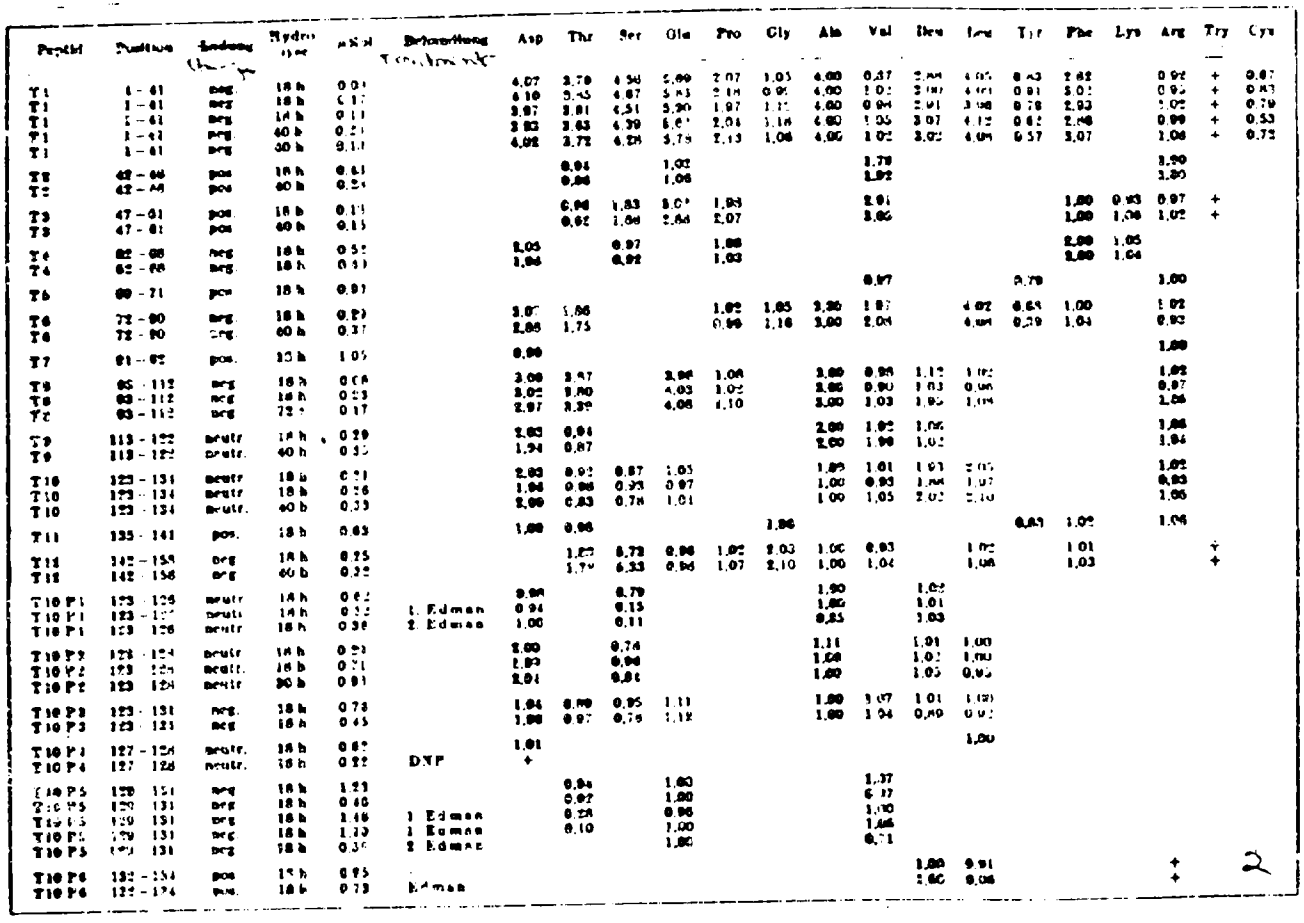
	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Vela.	Glu N	Phe	Ser	Glu N	Val	Try	Lys	Pro	Ser	Pro	Glu N	Val	Thr	Val	Arg	Phe	Pro	Asp	Ser	Asp	Phe	Lys
Ni 488	Glu N	Phe	Ser	Glu N	Val	Try	Lys	Pro	Ser	Pro	Glu N	Val	Ile	Val	Arg	Phe	Pro	Asp	Ser	Asp	Phe	Lys
Ni 492	Glu N	Phe	Ser	Glu N	Val	Try	Lys	Pro	Leu	Pro	Glu N	Val	Thr	Val	Arg	Phe	Pro	Asp	Ser	Asp	Phe	Lys
Ni 1186	Glu N	Phe	Ser	Glu N	Val	Try	Lys	Pro	Ser	Pro	Glu N	Val	Thr	Val	Arg	Phe	Ser	Asp	Ser	Asp	Phe	Lys
Ni 1188	Glu N	Phe	Ser	Glu N	Val	Try	Lys	Pro	Ser	Pro	Glu N	Val	Thr	Val	Arg	Phe	Ser	Asp	Ser	Asp	Phe	Lys

Abb. 1.

Figure 1

Peptide	Position	Bindung Lys	Reaktionszeit	pMol	Bemerkung	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Leu	Tyr	Lys	Arg	Trp	Cys
T1	1 - 41	arg	18 h	0.14		4.05	3.94	4.78	5.01	1.05	1.10	4.00	1.08	5.02	5.02	0.81	3.05		1.05	+	0.87
T1	1 - 41	arg	18 h	0.27		4.12	3.98	4.52	5.47	1.12	1.05	4.00	0.93	5.00	4.98	0.98	2.98		1.05	+	0.73
T1	1 - 41	arg	18 h	0.18		3.99	3.92	4.83	5.17	0.99	0.94	4.00	0.99	5.03	4.77	0.88	2.96		0.94	+	0.80
T1	1 - 41	arg	60 h	0.37		3.91	3.98	4.20	5.04	1.10	1.14	4.00	1.07	5.09	5.12	0.87	2.93		1.01	+	0.53
T1	1 - 41	arg	60 h	0.25		4.02	3.97	4.42	5.02	1.03	1.04	4.00	1.00	5.01	4.97	0.76	3.00		0.94	+	0.68
T2	42 - 46	pos	18 h	0.63			0.97		0.96				1.04						1.00		
T2	42 - 46	pos	60 h	0.42			0.99		0.99				2.05						1.00		
T3	47 - 61	pos	18 h	0.22			0.96	1.80	2.91	2.05			2.83				1.00	1.00	0.90	+	
T3	47 - 61	pos	60 h	0.27			0.85	1.84	2.90	2.01			3.01				1.00	0.87	1.05	+	
T4	62 - 86	arg	18 h	0.61		1.96		0.81		1.10							2.00	1.03			
T5	88 - 71	pos.	18 h	1.22									0.97			0.83			1.00		
T6	72 - 80	arg	18 h	0.28		2.04	1.91			0.96	1.03	3.00	1.02		2.97	0.72	0.96		1.05		
T6	72 - 80	arg	60 h	0.41		3.03	1.76			1.06	1.08	3.00	2.04		4.06	0.48	0.96		0.99		
T7	81 - 92	pro	18 h	1.23		1.08													1.00		
T8	93 - 112	arg	18 h	0.18		2.08	3.79		4.03	1.12		5.00	0.92	1.89	0.85				1.01		
T8	93 - 112	arg	18 h	0.29		3.08	3.82		3.85	1.02		5.00	0.97	1.08	1.07				1.03		
T8	93 - 112	arg	60 h	0.11		3.03	3.65		4.11	0.99		5.00	1.04	1.87	1.08				0.95		
T9	113 - 122	neutr.	18 h	0.42		1.88	0.96					2.00	1.87	1.00					1.81		
T9	113 - 122	neutr.	60 h	0.25		2.02	0.86					2.00	1.98	1.04					1.98		
T10	123 - 134	neutr.	18 h	0.11		1.83		0.91	1.02			1.00	0.94	1.83	2.06				1.01		
T11	135 - 141		18 h	0.43		1.00	0.99	0.88			1.78					0.80			1.08		
T12	142 - 150	arg	18 h	0.28			1.78	0.83	0.96	1.06	2.05	1.00	0.97		1.00		1.05				
T12	142 - 150	arg	60 h	0.17			1.65	0.79	1.03	1.14	2.12	1.00	1.04		1.99		0.94				
T1C1	1 - 8	arg	18 h	1.07				1.00								0.76					
T1C1	1 - 8	arg	18 h	1.44				1.00								0.92					
T1C5	1 - 10	arg	18 h	0.09			1.79	2.77	1.01	1.04				1.00		0.42	1.00				
T1C5	1 - 10	arg	18 h	0.24			1.78	2.63	1.16	1.10				1.01		0.86	1.00				
T1C8	3 - 10	neutr.	18 h	0.52			1.85	1.78	0.98	1.01				0.83			1.00				
T1C8	3 - 10	neutr.	18 h	0.25			1.88	1.62	0.93	1.02				0.87			1.00				
T1C8	3 - 10	neutr.	18 h	0.63			1.91	1.78	1.07	1.07				1.04			1.00				
T1C4	11 - 17	neutr.	18 h	0.88											0.98		1.00				
T1C4	11 - 17	neutr.	18 h	0.10											0.97		1.00				
T1C5	11 - 18	neutr.	18 h	0.52											0.84		1.03		1.00		
T1C6	11 - 86	arg	18 h	0.06		4.05	0.78	1.87	2.02		1.10	3.00	1.02	1.89	3.02		2.01			+	0.63
T1C7	11 - 84	arg	18 h	0.06		5.74	1.84	3.12	3.91		1.08	3.00	0.83	2.12	4.99		1.90			+	0.77
T1C7	11 - 84	arg	18 h	0.16		3.80	1.86	2.08	3.97		0.94	3.00	0.84	2.00	4.70		1.88			+	0.82
T1C8	18 - 33	arg	18 h	0.06		3.12			1.07			1.05		0.97	2.00						
T1C8	18 - 23	arg	18 h	0.05		1.08			0.99			1.02		0.95	2.00						
T1C8	18 - 23	arg.	18 h	0.04	1. Edman	0.90			1.02			0.10		1.01	2.00						
T1C8	18 - 23	arg	18 h	0.07	2. Edman	0.63			0.91					0.86	2.00						
T1C8	18 - 23	arg.	18 h	0.05	3. Edman	0.82			1.00					1.04	1.85						
T1C8	86 - 41	pos.	18 h	1.69			0.83		1.78			1.90							+		
T1C8	86 - 41	pos.	18 h	0.46			1.81		3.01			1.00							+		
T1C10	80 - 41	pos.	18 h	0.43					0.82			1.00							+		

Table 1. Amino acid composition of the peptides isolated from Mutant Ni-118 and localisation of the amino acid substitution



Peptid	Position	Ladung	Hydro-lyse	µMol	Bemerkung	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	neg.	18 h	0.01		4,07	3,78	4,56	5,89	2,07	1,05	4,00	0,87	2,44	4,05	0,43	2,82		0,92	+	0,87
T1	1 - 41	neg.	18 h	0,17		4,10	3,45	4,87	5,43	2,14	0,95	4,00	1,01	2,97	4,01	0,81	3,01		0,95	+	0,83
T1	1 - 41	neg.	14 h	0,11		3,87	3,81	4,51	5,90	1,97	1,11	4,00	0,94	2,91	3,98	0,78	2,93		1,02	+	0,79
T1	1 - 41	neg.	40 h	0,21		3,83	3,63	4,39	6,01	2,04	1,16	4,00	1,05	3,07	4,12	0,62	2,98		0,98	+	0,53
T1	1 - 41	neg.	40 h	0,10		4,02	3,72	4,29	5,79	2,13	1,06	4,00	1,02	3,02	4,04	0,57	3,07		1,06	+	0,72
T2	42 - 46	pos.	18 h	0,61			0,94		1,02				1,78						1,90		
T2	42 - 46	pos.	40 h	0,54			0,96		1,06				1,92						1,95		
T3	47 - 61	pos.	18 h	0,13			0,98	1,83	3,01	1,93			2,91				1,00	0,93	0,97	+	
T3	47 - 61	pos.	40 h	0,15			0,92	1,86	2,86	2,07			3,06				1,00	1,06	1,02	+	
T4	62 - 68	neg.	18 h	0,51		1,05		0,97		1,88							2,00	1,05			
T4	62 - 68	neg.	18 h	0,13		1,04		0,92		1,83							2,00	1,04			
T5	69 - 71	pos.	18 h	0,91									0,97			0,78			1,00		
T6	72 - 90	neg.	18 h	0,89		2,07	1,86			1,82	1,05	3,26	1,87		4,02	0,65	1,00		1,02		
T6	72 - 90	neg.	40 h	0,37		2,06	1,75			0,96	1,16	3,00	2,04		4,04	0,59	1,04		0,92		
T7	91 - 97	pos.	18 h	1,05		0,98													1,00		
T8	98 - 112	neg.	18 h	0,68		3,08	3,97		3,96	1,08		3,00	0,96	1,15	1,02				1,02		
T8	98 - 112	neg.	18 h	0,53		3,02	3,90		4,03	1,02		3,00	0,90	1,03	0,96				0,97		
T8	98 - 112	neg.	72 h	0,17		2,97	3,89		4,08	1,10		3,00	1,03	1,05	1,04				1,06		
T9	113 - 122	neutr.	18 h	0,29		2,03	0,94					2,00	1,02	1,06					1,06		
T9	113 - 122	neutr.	40 h	0,35		1,94	0,87					2,00	1,00	1,02					1,04		
T10	123 - 134	neutr.	18 h	0,21		2,03	0,92	0,87	1,05			1,89	1,01	1,01	2,05				1,02		
T10	123 - 134	neutr.	18 h	0,16		1,98	0,88	0,95	0,97			1,00	0,93	1,04	1,07				0,93		
T10	123 - 134	neutr.	40 h	0,33		2,00	0,83	0,78	1,01			1,00	1,05	2,02	2,10				1,06		
T11	135 - 141	pos.	18 h	0,63		1,00	0,96				1,86					0,83	1,02		1,04		
T11	142 - 156	neg.	18 h	0,25			1,03	0,73	0,98	1,02	2,03	1,00	0,83		1,02		1,01			+	
T11	142 - 156	neg.	40 h	0,22			1,79	0,53	0,96	1,07	2,10	1,00	1,04		1,08		1,03			+	
T10 P1	123 - 126	neutr.	18 h	0,62		0,94		0,79				1,90		1,02							
T10 P1	123 - 126	neutr.	14 h	0,32	1. Edman	0,94		0,15				1,86		1,01							
T10 P1	123 - 126	neutr.	18 h	0,36	2. Edman	1,00		0,11				0,85		1,03							
T10 P2	124 - 134	neutr.	18 h	0,53		2,00		0,74				1,11		1,01	1,00						
T10 P2	124 - 134	neutr.	18 h	0,31		1,93		0,98				1,04		1,02	1,00						
T10 P2	124 - 134	neutr.	96 h	0,81		2,01		0,81				1,00		1,05	0,95						
T10 P3	127 - 131	neg.	18 h	0,78		1,04	0,99	0,95	1,11			1,00	1,07	1,01	1,03						
T10 P3	127 - 131	neg.	18 h	0,45		1,00	0,97	0,79	1,12			1,00	1,04	0,99	0,97						
T10 P4	127 - 126	neutr.	18 h	0,62		1,01									1,00						
T10 P4	127 - 126	neutr.	18 h	0,22	DNP	+															
T10 P5	132 - 134	neg.	18 h	1,21			0,94		1,00				1,37								
T10 P5	132 - 134	neg.	18 h	0,46			0,92		1,00				1,37								
T10 P5	132 - 134	neg.	18 h	1,46	1 Edman		0,28		0,96				1,00								
T10 P5	132 - 134	neg.	18 h	1,19	1 Edman		0,10		1,00				1,46								
T10 P5	132 - 134	neg.	18 h	0,36	2 Edman				1,00				0,71								
T10 P6	132 - 134	pos.	18 h	0,95										1,00	0,91				+		2
T10 P6	132 - 134	pos.	18 h	0,73	Edman									1,00	0,08				+		

Table. 2. Amino acid composition of the peptides isolated from Mutant A-14 and localization of the amino acid substitution

Peptid	Position	Labeling	Hydrolysis	µMol	Remarks	Asp	Thr	Ser	Glu	Pro	Gly	-	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	arg.	18 h	[illegible]		4.01	[illegible]	[illegible]	[illegible]	2.03	1.05	4.00	[illegible]	[illegible]	[illegible]	0.80	2.45		1.02	+	0.83
T1	1 - 41	arg.	18 h	0.31		4.04	3.46	4.65	5.47	2.14	1.10	4.00	[illegible]	[illegible]	[illegible]	0.96	3.04		1.05	+	0.87
T1	1 - 41	arg.	24 h	0.25		3.95	3.73	4.44	5.59	2.06	[illegible]	4.00	0.24	2.46	4.18	0.48	[illegible]		0.83	+	0.73
T1	1 - 41	arg.	40 h	0.31		3.88	3.45	[illegible]	5.45	1.97	1.15	4.00	1.04	2.00	4.03	0.86	3.03		0.93	+	0.65
T1	1 - 41	arg.	40 h	0.13		4.06	3.39	4.31	5.75	2.01	1.06	4.00	1.01	2.02	4.10	0.78	2.94		1.01	+	0.38
T2	42 - 46	pos.	18 h	[illegible]			0.96		0.97				1.02						1.00		
T2	42 - 46	pos.	40 h	0.19			0.91		1.02				1.02						1.00		
T3	47 - 61	pos.	18 h	0.14				1.86	2.04	1.97			3.00	1.02			1.00	0.94	0.91	+	
T3	47 - 61	pos.	36 h	0.18				1.79	2.83	2.06			3.83	0.91			1.00	0.94	1.03	+	
T3	47 - 61	pos.	40 h	0.21				1.60	2.93	2.03			3.05	1.08			1.00	1.06	1.03	+	
T4	62 - 68	neg.	18 h	0.63		1.03		0.97		1.04							2.00	1.06			
T5	69 - 71	pos.	18 h	1.06									1.03			0.96			1.00		
T6	72 - 90	neg.	18 h	0.18		3.04	1.91			1.08	1.04	3.00	3.05		4.02	0.78	1.06		1.02		
T6	72 - 90	neg.	40 h	0.14		2.96	1.83			1.12	1.08	3.00	3.01		3.99	0.98	1.01		0.95		
T7	91 - 93	pos.	18 h	1.96		0.98													1.00		
T8	93 - 112	neg.	18 h	0.27		3.91	3.86		3.95	1.04		3.00	[illegible]	1.12	1.05				0.93		
T8	93 - 112	neg.	36 h	0.18		3.84	3.81		4.01	1.02		3.00	0.98	1.11	1.02				1.04		
T8	93 - 112	neg.	72 h	0.10		3.01	3.61		3.88	1.13		3.00	1.02	1.94	3.04				0.94		
T9	113 - 122	neutr.	18 h	0.33		3.04	0.83					2.00	1.93	1.05					1.95		
T9	113 - 122	neutr.	40 h	0.41		1.97	0.76					3.00	3.03	1.01					1.76		
T10	123 - 134	neutr.	18 h	0.27		3.86	0.93	0.89	0.93			1.00	0.85	1.84	1.97				1.93		
T10	123 - 134	neutr.	18 h	0.32		3.81	0.86	0.77	0.96			1.00	1.02	2.04	2.04				0.96		
T11	135 - 141	pos.	18 h	0.27		1.00	0.98				1.06					0.97	1.02		0.97		
T12	142 - 156	neg.	18 h	0.11			1.46	0.70	1.03	1.14	1.97	1.00	1.03		1.01		0.99			+	
T12	142 - 156	neg.	40 h	0.15			1.03	0.42	0.97	1.07	2.18	1.00	0.99		1.04		1.03			+	
T3C1	47 - 58	neutr.	18 h	0.48				0.88	2.06				1.00				[illegible]			+	
T3C2	47 - 57	pos.	18 h	0.22				1.86	3.00	1.98			1.00				1.00	0.96			
T3C3	58 - 67	pos.	18 h	1.46				0.84	0.93	2.00								1.06			
T3C4	58 - 61	pos.	18 h	0.91									1.05	0.97					1.00		
T3C4	58 - 61	pos.	18 h	0.33	1. Edman								1.15	0.88					1.00		
T3C4	58 - 61	pos.	36 h	0.26	2. Edman								1.10	0.79					1.00		
T3C4	58 - 61	pos.	18 h	0.28	3. Edman								0.81	0.15					1.00		

Table 3. Amino Acid Composition of peptides isolated from Mutant N-459 and localization of the Amino acid substitution.

Peptide	Position	Labeling	Hydrolysis Type	µMol	Remarks	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	neg	14 h	0.14		4.08	2.47	4.45	6.03	2.03	1.05	4.00	0.47	2.43	4.03	0.93	2.65		1.02	+	0.78
T1	1 - 41	neg	14 h	0.21		3.87	2.76	4.58	5.76	2.12	1.12	4.00	0.93	2.54	3.0	0.91	2.44		1.03	+	0.83
T1	1 - 41	neg	40 h	0.23		3.82	2.42	4.36	5.44	1.47	1.01	4.00	0.98	2.76	4.08	0.91	3.04		0.97	+	0.82
T1	1 - 41	neg	40 h	0.20		4.08	2.45	4.24	5.72	2.14	1.12	4.00	1.04	2.87	4.07	0.93	2.85		1.11	+	0.69
T1	1 - 41	neg	72 h	0.17		4.02	2.43	4.16	5.92	2.07	1.10	4.00	1.02	2.87	4.11	0.89	3.07		1.09	+	0.88
T2	42 - 48	pos	14 h	0.43			1.02		1.05				1.78						1.00		
T2	42 - 48	pos	40 h	0.61			0.88		0.99				2.09						1.00		
T3	47 - 81	pos	14 h	0.16			0.95	0.98	2.85	2.04			2.90		0.93		1.00	1.05	1.00	+	
T3	47 - 81	pos	14 h	0.27			0.97	0.60	3.06	2.10			2.97		0.95		1.00	0.98	0.96	+	
T3	47 - 81	pos	40 h	0.21			0.83	0.75	2.92	2.01			3.01		1.02		1.00	1.02	1.00	+	
T4	82 - 88	neg	14 h	0.43		1.07		0.94		1.10							2.00	0.97			
T5	89 - 71	pos	14 h	0.44									0.88			0.83			1.00		
T6	72 - 90	neg	14 h	0.14		2.02	1.88			1.15	1.04	3.00	1.98		4.05	0.87	1.02		0.96		
T6	72 - 90	neg	40 h	0.22		3.01	1.80			1.02	1.06	3.00	2.01		3.99	0.93	1.07		0.96		
T7	91 - 92	pos	14 h	0.83		1.02													1.00		
T8	93 - 112	neg	14 h	0.17		2.95	1.79		4.07	1.03		3.00	0.95	1.94	1.02				1.00		
T8	93 - 112	neg	14 h	0.23		3.02	1.44		3.92	1.12		3.00	1.02	1.12	0.97				1.02		
T8	93 - 112	neg	72 h	0.25		2.96	1.44		4.01	0.99		3.00	1.07	2.03	1.05				0.96		
T9	113 - 122	neutr	14 h	0.20		1.84	0.91					2.00	1.97	0.93					1.00		
T9	113 - 122	neutr	40 h	0.16		2.03	0.93					2.00	1.93	1.01					1.73		
T10	123 - 134	neutr	14 h	0.26		1.96	0.91	0.91	1.02			1.00	1.04	1.86	1.93				0.97		
T10	123 - 134	neutr	14 h	0.43		2.03	0.87	0.82	0.95			1.00	0.99	1.97	2.04				1.01		
T11	135 - 141	pos.	14 h	0.19		1.00	0.93				1.01					0.76	1.07		1.01		
T12	142 - 158	neg	14 h	0.16			1.96	5.74	0.97	1.07	2.06	1.00	0.96		0.92		1.02			+	
T12	142 - 158	neg	14 h	0.24			1.78	5.56	1.01	0.93	2.11	1.00	0.96		1.03		1.06			+	
T1C1	1 - 8	neg	20 h	0.87				1.00								0.96					
T1C2	1 - 10	neg	40 h	0.51			0.83	2.01	1.00	1.05				1.96		0.48	1.01				
T1C3	3 - 10	neutr	40 h	0.50			0.86	1.43	1.00	1.04				1.78			1.07				
T1C3	3 - 10	neutr	40 h	0.18	1 Edman		0.88	0.86	1.00	1.05				1.72			1.02				
T1C3	3 - 10	neutr	40 h	0.23	2 Edman		0.87	0.77	1.00	1.11				1.15			0.97				
T1C3	3 - 10	neutr	40 h	0.15	3 Edman		0.72	0.42	1.00	0.98				0.58			1.01				
T1C3s1	3 - 8	neutr	40 h	0.24			0.77	1.51		1.00				1.76							
T1C3s2	9 - 10	neutr	40 h	0.16					0.93								1.00				
T1C4	11 - 12	neutr.	20 h	0.67									0.93				1.00				
T1C5	11 - 17	neutr	20 h	0.32				1.76				1.00	0.91		1.03		0.99			+	
T1C6	18 - 23	neg	20 h	0.13		1.04			1.05	1.11		1.00		0.93	1.02						
T1C7	24 - 26	neutr	20 h	0.20		1.00								0.92	1.07						
T1C8	24 - 35	neutr	20 h	0.44		3.14	0.97		1.05		1.00	1.07		1.07	2.10		0.94				0.86
T1C8	24 - 35	neutr.	20 h	0.18		3.06	0.91		1.07		1.09	1.04		1.04	2.03		1.01				0.87
T1C9	27 - 35	neutr	20 h	0.09		2.02	0.93		1.06		1.06	1.02			0.86		1.00				0.43
T1C10	28 - 35	neutr.	20 h	0.30		1.98	0.94		1.00		1.10	0.96			1.01		0.92				
T1C11	36 - 38	neutr.	20 h	0.35			0.95		2.00												
T1C11	36 - 38	neutr.	20 h	0.49			0.95		2.00												
T1C12	39 - 41	pos.	20 h	0.76			0.92		0.87			1.00						+			
T1C12	39 - 41	pos.	20 h	0.79			0.96		1.01			1.00						+			
T1C12	39 - 41	pos.	20 h	0.52					0.45			1.00						+			
T2C1	47 - 82	neutr.	20 h	0.25				0.94	1.07				1.07				1.00			+	
T2C2	47 - 69	pos.	20 h	0.24			0.86	0.98	2.88	2.04			2.13		1.12		1.00	0.95		+	
T2C3	49 - 52	neutr.	20 h	0.10				0.78	1.00				1.00							+	
T2C4	49 - 87	pos.	20 h	0.12				0.82	1.43	2.05			1.00		0.87			+		+	
T3C5	80 - 81	pos	20 h	0.84			0.99		1.71	2.04			2.08		1.00			+	+	+	
T3C6	83 - 87	pos.	20 h	0.06					1.05	2.12					1.00			+			
T3C6	83 - 87	pos	20 h	0.16					1.01	2.05					1.00			+			
T3C6	83 - 87	pos.	20 h	0.10					0.93	2.13					1.00			+			
T3C7	87 - 91	pos	20 h	0.34					1.00	2.02			0.97		1.02			0.92			
T3C7	88 - 91	pos	20 h	0.21	1 Edman				1.00	1.95			1.01		1.03			0.21			
T3C7	87 - 91	pos	20 h	0.12	2 Edman				1.00	1.74			0.93		1.01			0.97			
T3C7	87 - 91	pos	20 h	0.12	3 Edman				0.91	1.72			0.98		0.31						
T3C7	87 - 91	pos	20 h	0.21	4 Edman				0.96	1.00			0.88		0.17						
T3C8	91 - 91	pos	20 h	0.16					0.97				1.75		1.01			0.88	0.97		
T3C8	88 - 91	pos.	20 h	0.41					0.00				1.00		1.08			+	+		
T3C9	92 - 91	pos	20 h	0.07					1.05				2.00						+		
T3C10	89 - 91	pos	20 h	0.30					0.91				1.00						+		
T3C10	92 - 91	pos	20 h	0.36					0.93				1.00						+		

Table 4. Amino acid composition of the peptides isolated from Mutant Ni-462 and localisation of the amino acid substitution.

Peptid	Position	Ladung	Hydrolyse	µMol	Bemerkung	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	arg.	18 h	0.17		3.82	3.[illegible]	4.[illegible]	5.[illegible]	2.11	1.05	4.00	0.78	2.03	4.07	0.[illegible]	3.05		1.06	+	0.87
T1	1 - 41	arg.	18 h	0.21		4.04	3.72	4.52	5.[illegible]	2.01	1.14	4.00	1.02	3.03	4.00	0.91	2.[illegible]		0.[illegible]	+	0.81
T1	1 - 41	arg.	18 h	0.20		3.93	3.[illegible]	4.[illegible]	5.[illegible]	1.97	1.02	4.00	0.98	2.[illegible]	4.[illegible]	0.[illegible]	2.98		1.02	+	0.75
T1	1 - 41	arg.	40 h	0.35		3.92	3.41	4.00	4.[illegible]	2.10	1.21	4.00	1.07	3.11	4.17	0.[illegible]	3.01		1.02	+	0.82
T1	1 - 41	arg.	40 h	0.26		4.12	3.[illegible]	4.[illegible]	5.[illegible]	2.07	1.03	4.00	1.[illegible]	3.03	4.08	0.[illegible]	3.04		0.94	+	0.57
T2	42 - 48	pos.	18 h	0.[illegible]			0.[illegible]		0.[illegible]				1.[illegible]						1.00		
T2	42 - 48	pos.	40 h	0.42			0.79		1.08				1.[illegible]						1.00		
T3	47 - 81	pos.	18 h	0.24			0.[illegible]	1.[illegible]	2.[illegible]	2.12			2.[illegible]				1.00	0.[illegible]	0.[illegible]	+	
T3	47 - 81	pos.	18 h	0.[illegible]			0.[illegible]	1.[illegible]	2.[illegible]	1.[illegible]			3.02				1.[illegible]	0.[illegible]	1.[illegible]	+	
T3	47 - 81	pos.	40 h	0.[illegible]			0.[illegible]	1.83	2.92	2.05			3.10				1.00	0.97	1.01	+	
T4	82 - 84	neg.	18 h	0.[illegible]		2.03		1.96									2.00	0.99			
T4	82 - 84	neg.	18 h	0.91		1.98		1.73									2.00	1.10			
T4	82 - 84	neg.	40 h	0.80		2.01		1.68									2.00	1.03			
T5	99 - 71	pos.	18 h	1.08									0.96			0.[illegible]			1.01		
T6	72 - 90	neg.	18 h	0.20		2.12	1.90			1.06	1.12	3.00	1.[illegible]		4.02	0.76	0.93		1.02		
T6	72 - 90	neg.	40 h	0.17		2.00	1.78			1.02	1.04	3.00	2.02		4.04	0.87	1.01		1.01		
T7	91 - 92	pos.	18 h	0.[illegible]		0.91													1.00		
T8	93 - 112	neg.	18 h	0.00		2.84	2.81		4.01	0.87		3.00	0.[illegible]	1.[illegible]	1.[illegible]				1.01		
T8	93 - 112	neg.	18 h	0.14		2.93	2.[illegible]		4.04	1.07		3.00	0.[illegible]	1.07	0.95				0.97		
T8	93 - 112	neg.	72 h	0.23		2.94	2.81		3.97	1.10		3.00	0.97	1.[illegible]	1.09				1.05		
T9	113 - 128	neutr.	18 h	0.46		2.06	0.92					2.00	1.49	0.91					1.79		
T9	113 - 128	neutr.	40 h	0.57		1.84	0.89					2.00	2.01	1.04					1.93		
T10	129 - 134	neutr.	18 h	0.14		2.05	0.96	0.89	0.97			1.00	0.88	1.78	1.89				1.02		
T10	129 - 134	neutr.	40 h	0.22		2.01	0.85	0.75	1.03			1.00	1.04	1.97	2.05				0.96		
T11	135 - 141	pos.	18 h	0.27		1.00	0.95				1.66					0.83	0.90		0.88		
T12	142 - 158	neg.	18 h	0.13			1.01	1.67	1.08	0.97	2.02	1.00	0.03		1.05		1.01			+	
T12	142 - 158	neg.	40 h	0.21			1.04	1.29	0.99	1.06	2.00	1.00	1.02		1.02		0.96			+	
T4	82 - 83	neg.	18 h	0.23		2.02		1.86									2.03	1.00			
T4	82 - 84	neg.	18 h	0.27	1. Edman	1.94		1.73									1.14	1.02			
T4	82 - 86	neg.	18 h	0.19	2. Edman	1.87		1.09									1.06	1.00			
T4	82 - 86	neg.	18 h	0.26	3. Edman	1.55		0.96									1.08	1.00			

Table 5. Amino acid composition of peptides isolated from Mutant Ni-1196 and localisation of the amino acid substitution.

Peptid	Position	Ladung	Hydrolyse	µMol	Bemerkung	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	arg.	18 h	0.23		4.06	3.82	4.53	4.04	2.02	1.03	4.00	1.03	2.03	4.06	0.85	2.84		0.91	+	0.66
T1	1 - 41	arg.	18 h	0.17		4.13	3.75	4.66	5.[illegible]	1.96	1.11	4.00	0.97	2.94	4.13	0.76	3.04		1.02	+	0.81
T1	1 - 41	arg.	18 h	0.12		3.94	3.[illegible]	4.72	5.[illegible]	2.16	1.02	4.00	0.91	2.85	4.01	0.92	2.75		1.06	+	0.73
T1	1 - 41	arg.	40 h	0.18		3.87	3.34	4.[illegible]	5.[illegible]	2.12	1.14	4.00	1.07	3.07	4.11	0.73	3.08		0.97	+	0.44
T1	1 - 41	arg.	40 h	0.21		4.02	3.36	4.34	5.[illegible]	2.07	1.07	4.00	1.11	3.00	4.03	0.84	3.12		0.99	+	0.62
T2	42 - 48	pos.	18 h	0.34			0.[illegible]		1.08				1.[illegible]						1.00		
T2	42 - 48	pos.	40 h	0.45			0.[illegible]		0.[illegible]				2.05						1.00		
T3	47 - 81	pos.	18 h	0.25			0.[illegible]	1.83	2.87	2.01			2.94				1.00	1.08	1.01	+	
T3	47 - 81	pos.	40 h	0.32			0.[illegible]	1.76	2.[illegible]	2.06			3.03				1.00	1.02	0.96	+	
T4	82 - 86	neg.	18 h	0.42		2.02		1.92									2.00	1.07			
T4	82 - 86	neg.	18 h	0.25		1.96		1.91									2.00	1.04			
T4	82 - 86	neg.	40 h	0.32		1.96		1.88									2.00	0.96			
T5	99 - 71	pos.	18 h	0.83									1.01			0.[illegible]			1.00		
T6	72 - 90	neg.	18 h	0.13		2.[illegible]	1.[illegible]			1.[illegible]	1.[illegible]	3.00	1.[illegible]		3.[illegible]	0.[illegible]	1.00		1.[illegible]		
T6	72 - 90	neg.	18 h	0.28		2.96	1.90			1.[illegible]	0.97	3.00	2.02		4.[illegible]	0.83	1.[illegible]		0.[illegible]		
T6	72 - 90	neg.	40 h	0.19		3.01	1.73			1.16	1.11	3.00	2.06		4.06	0.[illegible]	0.[illegible]		1.[illegible]		
T7	91 - 92	pos.	18 h	1.03		1.04													1.00		
T8	93 - 112	neg.	18 h	0.[illegible]		2.[illegible]	2.[illegible]		4.[illegible]	1.[illegible]		3.[illegible]	1.[illegible]	1.[illegible]	1.[illegible]				1.[illegible]		
T8	93 - 112	neg.	18 h	0.16		3.13	2.[illegible]		4.16	0.97		3.00	0.95	1.[illegible]	0.[illegible]				0.[illegible]		
T8	93 - 112	neg.	72 h	0.24		3.05	2.57		4.01	1.13		3.00	1.06	1.97	1.00				1.04		
T9	113 - 128	neutr.	18 h	0.36		1.95	0.91					2.00	1.38	0.87					1.84		
T9	113 - 128	neutr.	40 h	0.54		2.00	0.97					2.00	2.07	0.96					2.04		
T10	129 - 134	neutr.	18 h	0.43		1.80	0.93	0.90	1.02			1.00	1.02	1.[illegible]	2.03				0.91		
T10	129 - 134	neutr.	40 h	0.62		2.02	0.78	0.78	1.01			1.00	1.01	2.05	2.08				1.02		
T11	135 - 141	pos.	18 h	0.66		1.00	0.96				1.74					0.77	1.08		1.08		
T11	135 - 141	pos.	40 h	0.53		1.00	0.86				1.80					0.[illegible]	1.01		1.02		
T12	142 - 158	neg.	18 h	0.14			1.00	1.[illegible]	1.05		2.07	1.00	0.87		2.01		1.00			+	
T12	142 - 158	neg.	18 h	0.12			1.[illegible]	1.[illegible]	0.[illegible]		2.09	1.00	0.[illegible]		1.[illegible]		1.05			+	
T12	142 - 158	neg.	40 h	0.21			1.71	1.[illegible]	0.[illegible]		1.[illegible]	1.00	1.[illegible]		2.[illegible]		0.[illegible]			+	
T4	82 - 83	neg.	18 h	0.61	1. Edman	2.01		1.78									1.11	1.00			
T4	82 - 84	neg.	18 h	0.80	2. Edman	1.[illegible]		1.[illegible]									1.[illegible]	1.00			
T4	82 - 86	neg.	18 h	0.70	3. Edman	1.73		1.[illegible]									1.[illegible]	1.00			
T12	142 - 158	neg.		0.48	Chase A 5'		1.00					0.[illegible]									
T12	142 - 158	neg.		0.61	Chase A 30'		1.00					0.[illegible]			0.[illegible]						
T12	142 - 158	neg.		0.48	Chase A 2 h		1.00					1.[illegible]			1.[illegible]						

Table 6. Amino acid composition of the peptides isolated from Mutant Ni-1688 and localisation of the amino acid substitution.

Probe	Position	Isolierung	Hydrolyse	pH	Bemerkung	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	neg	14 h	0.11		4.05	3.78	3.62	6.02	2.02	1.03	4.00	0.94	3.07	6.01	0.87	2.94		1.02	+	0.66
T1	1 - 41	neg	14 h	0.20		4.07	3.82	3.54	5.87	2.11	0.99	4.00	1.05	2.94	6.05	0.88	2.95		1.05	+	0.73
T1	1 - 41	neg	40 h	0.18		3.97	3.81	3.29	5.73	2.03	1.13	4.00	1.15	2.93	6.07	0.72	2.92		0.96	+	0.66
T1	1 - 41	neg	40 h	0.16		4.02	3.58	3.34	5.82	2.07	1.08	4.00	1.04	3.07	6.12	0.86	2.83		1.04	+	0.46
T2	42 - 46	pos.	18 h	1.14			1.04		1.04				1.65						+		
T3	47 - 61	pos	18 h	0.24			0.89	2.10	2.87	2.20			2.84				3.00	+	+	+	
T3	47 - 61	pos.	14 h	0.49			0.86	2.08	2.93	2.15			3.00				3.00	+	+	+	
T4	62 - 68	neg	14 h	0.12		1.97		1.11		1.00							2.00	+			
T5	69 - 71	pos.	18 h	0.49									1.00			0.45			+		
T6	72 - 80	neg	18 h	0.11		3.10	2.50			1.28	1.00	3.50	3.70		4.00	0.35	1.60		+		
T7	81 - 82	pos.	18 h	0.30		1.00													+		
T8	83 - 112	neg	18 h	0.41		5.84	3.75		3.80	1.02		3.22	0.83	0.89	1.00				+		
T9	113 - 118	neutr	14 h	0.50		2.00	1.02					1.78	1.92	0.94					+		
T9	119 - 122	neutr	14 h	0.16		1.00	0.81					2.25	2.33	1.10					+		
T10	123 - 134	neutr.	18 h	0.31		2.00	0.85	1.00	1.03			2.11	1.08	1.95	2.04				+		
T11	135 - 141	pos	18 h	0.46		1.00	1.01	1.02			1.04					0.78			+		
T12	142 - 156	neg	14 h	0.08			0.87	5.50	1.07	1.04	2.00	1.00	1.00	0.92	0.92		0.84			+	
T12	142 - 156	neg	18 h	0.20			0.86	5.95	0.93	1.02	1.90	1.00	0.73	0.91	0.96		0.90			+	
T1C1	1 - 2	neg	14 h	0.13				1.00								0.86					
T1C2	1 - 10	neg	14 h	0.34			1.92	2.84	2.03	1.09				0.93		0.86	1.00				
T1C2	1 - 10	neg	18 h	0.42			1.88	2.87	0.87	1.11				1.02		0.83	1.00				
T1C3	3 - 10	neutr	14 h	0.62			2.03	1.91	1.07	1.14				1.00			1.00				
T1C3	3 - 10	neutr.	14 h	0.24			1.80	1.73	1.03	1.05				0.98			1.00				
T1C4	11 - 12	neutr.	14 h	0.54									0.83				1.00				
T1C4	11 - 12	neutr	14 h	0.45									0.97				1.00				
T1C5	11 - 17	neutr	18 h	0.46				1.78				1.00	0.90		1.03		1.04			+	
T1C6	13 - 17	neutr	18 h	0.22				0.91				1.00			2.07					+	
T1C6	13 - 17	neutr	14 h	0.83				0.88				1.00			1.99					+	
T1C6	13 - 17	neutr.	16 h	0.18	1. Edman			0.83				1.00			1.24					+	
T1C6	13 - 17	neutr.	18 h	0.21	2. Edman			0.81				1.00			1.18					+	
T1C6	13 - 17	neutr.	18 h	0.20	3. Edman			0.12				1.00			0.78					+	
T1C6	13 - 17	neutr	18 h	0.10	DNP										+						
T1C6	13 - 17	neutr			C' ase A: 10'							1.00			0.32					-	
T1C6	13 - 17	neutr.			C' ase A: 60'			0.91				1.00			0.60					-	
T1C7	18 - 25	neg	14 h	0.41		1.02			0.97	1.07		1.03		0.93	1.00						
T1C8	18 - 35	neg.	18 h	0.13		3.09	0.82		1.93	1.10	0.97	2.00		1.79	2.64		1.02				0.86
T1C9	26 - 35	neutr.	18 h	0.06		3.06	0.70		0.99		1.06	1.00		0.84	2.07		1.04				0.72
T1C10	30 - 35	neutr.	18 h	0.07		0.96			0.96		1.06	1.04			1.02		1.00				
T1C11	32 - 35	neutr.	18 h	0.51		1.08			1.01		1.11						1.00				
T1C12	36 - 38	pos.	18 h	0.36			1.04		2.00												
T1C13	36 - 41	pos.	18 h	0.87			0.98		3.05			1.00							+		
T1C14	39 - 41	pos	18 h	0.85					0.95			1.00							+		
T1EC4	155 - 159	neutr.	18 h	0.20			0.38	1.90		0.99	1.00	1.00		0.88							
T1EC4	168 - 186	neutr.			AH' ase			+						1.00							
T1EC4	155 - 159	neutr.			DNP									+							

Table 7. Amino acid composition of peptides isolated from Mutant Ni-220/4 and localisation of the amino acid substitution.

Peptid	Position	Ladung	Hydrolyse	pMol	Bemerkung	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	neg	18 h	0.22		4.02	3.73	3.72	5.74	2.05	1.04	4.00	1.11	2.96	5.05	0.88	2.91		0.95	+	0.72
T1	1 - 41	neg	18 h	0.22		4.07	3.82	3.54	6.04	1.90	1.36	4.00	0.95	3.03	4.82	0.91	2.92		0.92	+	0.62
T1	1 - 41	neg	40 h	0.18		4.12	3.84	3.84	5.93	2.14	1.15	4.00	1.06	3.11	5.14	0.78	2.75		1.02	+	0.51
T1	1 - 41	neg	40 h	0.23		5.04	3.83	3.85	5.82	2.08	1.06	4.00	1.02	3.02	5.10	0.75	2.91		1.04	+	0.54
T2	42 - 46	pos.	18 h	0.48			0.98		1.00				1.72						+		
T3	47 - 81	pos	18 h	0.36			0.91	1.92	3.01	2.06			2.91				1.04	+	+	+	
T4	82 - 86	neg.	18 h	0.17		2.00		1.74		0.75							1.00	+			
T5	88 - 71	pos.	18 h	0.61									1.00			0.48			+		
T6	72 - 90	neg	18 h	0.82		2.00	2.12			0.93	0.96	3.65	1.94		4.00	0.98	1.00		+		
T7	91 - 92	pos.	18 h	0.21		1.00													+		
T8	93 - 115	neg	18 h	0.10		3.10	3.96		4.02	0.87		3.00	1.20	0.95	0.96				+		
T9	113 - 122	neutr.	18 h	0.10		2.01	0.97					2.00	1.78	0.96					+		
T9	113 - 122	neutr.	15 h	0.28		2.00	1.02					1.86	1.86	1.02					+		
T10	123 - 134	neutr	18 h	0.13		1.98	0.98	0.94	0.99			1.00	1.03	2.03	3.03				+		
T11	135 - 141	pos	18 h	1.15		1.00	1.03	1.02			1.76					0.98			+		
T12	142 - 158	neg.	18 h	0.08			1.87	5.42	1.08	1.09	2.19	1.00	0.65		0.91		0.93			+	
T1C1	1 - 2	neg.	18 h	0.12				1.00								0.94					
T1C2	1 - 10	neg.	18 h	0.47			1.88	3.78	1.01	1.09				0.97		0.99	1.00				
T1C3	3 - 10	neutr.	18 h	0.28			1.92	1.78	1.04	1.07				0.98			0.96				
T1C3	3 - 10	neutr.	18 h	0.40			1.87	1.85	0.98	1.02				1.04			1.00				
T1C4	11 - 12	neutr.	18 h	0.67									0.92				1.00				
T1C4	11 - 12	neutr.	18 h	0.49									0.86				1.00				
T1C5	13 - 17	neutr.	18 h	0.25				0.00				1.00			1.93					+	
T1C5	13 - 17	neutr.	18 h	0.42				0.21				1.00			2.00					+	
T1C5	13 - 17	neutr.	18 h	0.10	C'ase A: 10'							1.00			0.18					+	
T1C5	13 - 17	neutr.	18 h	0.16	C'ase A: 60'							1.00			0.73					+	
T1C6	18 - 23	neg.	18 h	0.17		0.99			1.03	1.08		0.96		0.87	1.00						
T1C7	24 - 35	neutr.	18 h	0.12		2.03	0.86		1.06		1.12	1.00		0.77	2.10		1.01				0.84
T1C8	36 - 41	pos.	18 h	0.62			0.92		2.06			1.00							+		
T1C9	30 - 41	pos.	18 h	0.34					0.82			1.00							+		

Table 8. Amino acid composition of peptides isolated from Mutant Ni-2239 and localisation of the amino acid substitution.

Peptide	Position	Labeling	Hydrolysis	μMol	Behandlung TREATMENT	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	arg	18 h	0.17		4.05	3.82	4.65	5.95	1.94	1.05	4.00	0.97	2.84	4.04	0.87	3.02		1.93	+	0.53
T1	1 - 41	arg	18 h	0.23		4.05	3.75	4.57	6.02	2.07	1.12	4.00	1.05	2.93	3.93	0.95	2.41		0.94	+	0.64
T1	1 - 41	arg	40 h	0.25		4.12	3.57	4.23	5.73	2.05	1.02	4.00	1.12	3.07	4.10	0.93	2.45		0.94	+	0.73
T1	1 - 41	arg	40 h	0.19		4.04	3.64	4.54	5.69	2.15	0.94	4.00	1.03	3.01	4.13	0.73	2.96		1.02	+	0.54
T2	42 - 46	pos.	18 h	1.80			0.95		1.00				1.08						+		
T3	47 - 61	pos.	18 h	1.09			0.86	1.90	3.02	2.10			3.10				1.00	+	+	+	
T4	62 - 64	arg	18 h	0.62		1.01		0.91		1.05							0.00	+			
T5	69 - 71	pos.	18 h	0.35									1.00			0.76			+		
T6	72 - 80	arg	18 h	0.40		2.00	2.06			1.11	1.12	3.00	2.07		3.00	0.34	1.05		+		
T7	81 - 82	pos.	18 h	0.76		1.00													+		
T8	83 - 112	arg	14 h	0.24		2.92	3.77		5.84	1.03		3.00	0.94	0.91	1.07				+		
T9	113 - 122	neutr.	18 h	1.54		2.00	0.97					3.10	1.93	0.2					+		
T10	123 - 134	neutr.	14 h																		
T11	135 - 161	pos.	14 h	0.72		1.00	0.92	0.97			1.04					0.45			+		
T12	162 - 158	arg	18 h	0.44			1.87	5.80	0.94	0.99	1.09	1.00	0.49		0.92		0.89			+	

Table 9. Amino acid composition of the peptides isolated from mutant Ni-2516.

Peptide	Position	Labeling	Hydrolysis	μMol	Behandlung TREATMENT	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ileu	Leu	Tyr	Phe	Lys	Arg	Try	Cys
T1	1 - 41	arg	18 h	0.19		3.95	3.96	4.53	6.04	2.13	1.04	4.00	1.01	2.87	4.07	0.74	2.87		1.02	+	0.71
T1	1 - 41	arg	18 h	0.24		4.03	3.78	4.67	6.04	2.05	1.11	4.00	0.94	2.92	3.95	0.91	3.0		1.02	+	0.63
T1	1 - 41	arg	40 h	0.17		4.11	3.62	4.33	5.93	2.14	1.06	4.00	1.06	3.04	4.10	0.82	2.92		0.98	+	0.54
T1	1 - 41	arg	40 h	0.15		4.05	3.68	4.39	5.73	2.09	1.14	4.00	1.10	3.05	4.12	0.53	2.93		1.01	+	0.70
T2	42 - 46	pos.	40 h	0.94			0.94		1.00				1.72						+		
T3	47 - 61	pos	40 h	0.60			0.88	1.97	3.00	2.12			2.94				1.00	+	+	+	
T4	62 - 64	arg.	40 h	1.64		2.00		0.94		1.04							1.03	+			
T5	69 - 71	pos	40 h	1.40									1.00			0.90			+		
T6	72 - 80	arg	40 h	0.94		3.01	1.98			1.08	1.95	3.00	2.10		3.05	0.52	1.07		+		
T7	81 - 82	pos	40 h	0.76		1.00													+		
T8	83 - 112	arg	40 h	0.10		3.18	3.98		5.85	1.05		3.00	1.00	0.77	1.24				+		
T9	113 - 122	neutr.	40 h	1.60		2.00	1.04					3.00	1.99	1.17					+		
T10	123 - 134	neutr	40 h	0.28		1.75	0.91	0.90	1.05			1.00	1.08	1.82	1.76				+		
T11	135 - 161	neutr.	40 h	1.14		1.00	0.98	0.93			1.97					0.59			+		
T12	162 - 158	arg	40 h	0.40			1.99	5.72	1.00	1.09	2.15	1.06	0.93		1.15		1.00			+	

Table 10. Amino acid composition of the peptides isolated from mutant Ni-2519.

Mutant	Substitution	Position
flavum	Asp → Ala	19
necans	Phe → Leu	10
	Ala → Val	19
	Ser → Phe	138
subflavescens	Leu → Phe	10
rubrescens	Leu → Phe	10
	Val → Asp	19
CP 415	AspN → Lys	140
Ni 118	Pro → Leu	20
A 14	Ileu → Thr	129
Ni 458	Thr → Ileu	59
Ni 462	Thr → Ileu	5
	Ser → Leu	55
Ni 1186	Pro → Ser	63
Ni 1688	Pro → Ser	63
	Pro → Leu	156
Ni 2204	Ser → Leu	15
	Thr → Ileu	153
Ni 2239	Ser → Leu	15
Ni 2516	-	-
Ni 2519	-	-

Table 11. Nature and position of amino acid substitutions of mutants described in this and the preceding investigation (1). The mutants differ from the parent virus by the substitutions indicated.

Mutants	5	10	15	19	20	55	59	63	129	138	140	153	156
Vulgare	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Ileu	Ser	AspN	Thr	Pro
flavum	Thr	Phe	Ser	Ala	Pro	Ser	Thr	Pro	Ileu	Ser	AspN	Thr	Pro
necans	Thr	Leu	Ser	Val	Pro	Ser	Thr	Pro	Leu	Phe	AspN	Thr	Pro
subflavescens	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Ileu	Phe	AspN	Thr	Pro
rubrescens	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Ileu	Phe	AspN	Thr	Pro
CP 415	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Ileu	Ser	Lys	Thr	Pro
Ni 118	Thr	Phe	Ser	Asp	Leu	Ser	Thr	Pro	Ileu	Ser	AspN	Thr	Pro
A 14	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Thr	Ser	AspN	Thr	Pro
Ni 458	Thr	Phe	Ser	Asp	Pro	Ser	Ileu	Pro	Thr	Ser	AspN	Thr	Pro
Ni 462	Ileu	Phe	Ser	Asp	Pro	Leu	Thr	Pro	Thr	Ser	AspN	Thr	Pro
Ni 1186	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Ser	Thr	Ser	AspN	Thr	Pro
Ni 1688	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Ser	Thr	Ser	AspN	Thr	Leu
Ni 2204	Thr	Phe	Leu	Asp	Pro	Ser	Thr	Pro	Thr	Ser	AspN	Ileu	Pro
Ni 2239	Thr	Phe	Leu	Asp	Pro	Ser	Thr	Pro	Thr	Ser	AspN	Thr	Pro
Ni 2516	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Thr	Ser	AspN	Thr	Pro
Ni 2519	Thr	Phe	Ser	Asp	Pro	Ser	Thr	Pro	Thr	Ser	AspN	Thr	Pro

Table. 12. Primary structures of the mutants described in this and the preceding investigation (1). Other than the positions indicated, the amino acid sequences of the mutants are the same as that of the TMV strain vulgare(15).